Serum Leptin Levels in Rheumatoid Arthritis and Relationship with Disease Activity

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a long-lasting autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. Often, symptoms come on gradually over weeks to months. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present.

Objective: To study leptin levels in rheumatoid arthritis and relationship between these levels and disease activity.

Conclusion: Blocking the key signal pathways of leptin and inhibiting leptin activity, such as with leptin antagonists, may be a promising way for the therapeutic potential of RA at risk of detrimental effects. Hence, further understanding of the mechanism of leptin would be advantageous in the future in RA treatment.

Keywords: Serum leptin levels, Rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory systemic disorder that is characterized by polyarthritis with often-progressive joint damage and disability, immunologic abnormalities, increased comorbidity, and premature mortality. It affects the joints, connective tissues, muscle, tendons, and fibrous tissue. It tends to strike during the most productive years of adulthood, between the ages of 20 and 40, and is a chronic disabling condition often causing pain and deformity. Within 10 years on onset, at least 50% of patients, in developed countries, are unable to hold down a full-time job. The prevalence of RA varies between 0.3% and 1% and is more common in women and in developed countries [(2)]. RA is characterized by high concentrations of pro-inflammatory cytokines. The excessive production of inflammatory cytokines not only with joint disease activity and progression, but also with a loss of body cell mass, known as rheumatoid cachexia [(1)].

Leptin is a peptide hormone synthesized mostly by white adipose tissue cells. Endothelial cells, T-lymphocytes, bone marrow cells and platelets contribute to its production. Leptin was initially described, as a hormone that regulates food intake and energy balance and it is a mediator of the inflammatory process. In addition, it interacts with the immune system response and its concentration increases during infection and inflammation [(3)].

During acute inflammation, pro-inflammatory cytokines increase circulating leptin concentration and leptin in turn potentiates cytokine release from monocytes and macrophages. The involvement of leptin in regulating immune function in human is strongly sustained by the increased incidence of severe infection in subjects with genetic leptin deficiency. Leptin has been recently considered important factor in RA pathogenesis [(4)]. Therefore, we aimed to study leptin levels in rheumatoid arthritis and relationship between these levels and disease activity.

Epidemiology:

The prevalence of RA varies between 0.3% and 1% [(2)]. It affects between 5 and 50 per 100.000 people each year [(5)]. Women are affected three to five times as often as men. The age at which the disease most commonly starts in women is between 40 and 50 years of age, and for men somewhat later [(6)].

Genetics:

Half of the risk for RA is believed to be genetic. It is strongly associated with the major histocompatibility complex (MHC) antigen HLA-DRBI (most specifically the shared epitope alleles, including 0401 and born 0404), and the genes PTPN22 and PADI4. Hence, family history is an important risk factor [(7)]. Inheriting the PTPN22 gene has been shown to double a person's susceptibility to RA. PADI4 has been identified as a major risk factor in people of Asian descent, but not in those of European descent [(8)].

Pathogenesis:

RA is an autoimmune disease characterized by infiltration of inflammatory cells in the synovial membrane of affected joints, leading to pannus formation. The precise induction and progression of this process remains unclear. However, the presence of T and B cell infiltrates in the inflamed synovial tissue is a consistent histological finding in RA [(9)]. Genetic studies have demonstrated that RA is strongly correlated with the MHC class II antigen HLA-DR4. The main function of HLA-DR molecules is to present antigenic peptides to T cells. The synovial membrane contains a large number of CD4 helper T cells. Furthermore, auto-antibodies such as RF: appear to be associated with more aggressive articular disease [(10)].

Received:22 /10 /2020
Accepted:11 /2/2020

Role of T cells in the pathogenesis of RA:

The role of T cells in the pathogenesis of RA is well established. The RA disease process is thought to be dependent on a tri-molecular complex consisting of antigenic peptides, T- cell receptors and HLA-DR4. In RA, the antigen presenting cells (APCs) take up one or more unknown antigens and process them into peptides that are inserted into the groove of HLA-DR4 located on the surface of the APC. T cells with the appropriate T cell receptors then engage with this complex (forming a tri-molecular complex) to become activated. Subsequently, this causes a number of events including the production of IL-2, which leads to the clonal expansion of T cell [(9)]. Other changes occur to the T cells, the T cells begin to grow larger and start to express a number of surface molecules, such as CD69, tumour necrosis factor-α (TNF-α) and RANKL [(10)].

The pathogenesis of RA can be subdivided into different phases [(11)]:

1) An initiation phase with no clinical evidence of disease.
2) An early inflammatory phase, which leads to clinical manifestations.
3) A destructive phase accompanied by erosions and progression of the disease.
4) A perpetuating phase characterized by irreversible joint destruction.

a- Initiation phase: In RA, self-sustaining synovitis is probably triggered by presentation of a relevant antigen to a susceptible host. So far, the causative agent remains unknown. Retroviruses super antigens or antigens, such as mycobacterial heat shoch protein (HSP), mimicking host specific proteins and mycoplasma are candidate exogenous agents [(12)].

b- Early inflammatory phase: After an antigenic stimulation, T cells migrate and accumulate in the synovium. Activated T cells produce cytokines, such as IFN-y that subsequently stimulate monocyte/macrophage accumulation in the synovial lining. These activated macrophages release pro-inflammatory mediators in the synovium, which induce the expression of adhesion molecules on endothelial cells, thus enhancing the trans endothelial migration of lymphocytes and macrophages [(13)].

c- Irreversible Destruction of Cartilage: Synovial lining cells proliferate and organize in an invasive front of about 5 to 10 cell layers thick, irreversibly invading the underlying cartilage and bone [(14)].

Clinical findings and diagnosis of rheumatoid arthritis:

The initial symptoms of RA (frequently articular) typically can present by one of the two ways; with a slow insidious onset or an explosive sudden onset. In some individuals, fatigue, malaise, puffy hands, diffuse musculoskeletal pains or morning stiffness may be the first nonspecific complaints with synovitis occurring late. Most RA patients experience generalized malaise or fatigue and the hallmark of the disease is a chronic, symmetric polyarthritis that typically affects the hands, wrists and feet initially and later may involve any joint lined by a synovial membrane. RA primarily affects joints, but it also affects other organs in more than 15-25% of individuals [(15)].

Neurological:

Peripheral neuropathy and mononeuritis multiplex may occur. The most common problem is carpal tunnel syndrome caused by compression of the median nerve by swelling around the wrist leading to painful movement of the wrest with tingling sensation. Atlanto- axial subluxation can occur [(16)].

Constitutional symptoms:

Fatigue, low-grade fever, malaise, morning stiffness, loss of appetite and loss of weight are common systemic manifestations seen in people with active RA [(16)].

Laboratory findings of RA:

Many physicians depend on symptoms and signs of the disease activity but it is useful to have laboratory confirmation, to assess the disease activity, to follow up the disease, and to monitor the response to treatment [(17)]. These include:

1- Erythrocyte sedimentation rate (ESR): ESR is rapid but non-specific for any inflammatory condition, infection or malignancy. ESR is more useful in following the disease course and response to therapy [(17)].

2- C-Reactive protein (CRP): It is one of acute phase proteins, which frequently, is elevated in patients with RA. Serial measurements of CRP levels are very useful in assessing exacerbation and remissions. CRP is a better indicator of disease activity than ESR [(18)].

3- Complete blood picture: Which may show anemia, leucopenia, thrombocytosis or thrombocytopenia [(18)].

4- Synovial fluid analysis: An inflammatory synovial fluid is straw colored and slightly cloudy and contains many flecks of fibrin. A clot forms in fluid left standing at room temperature. IgG in synovial fluid may approach serum concentration. Complement C2 and C4 levels are depressed but C3 level can be normal [(18)].

5- Auto-antibodies associated with RA:

(A)Rheumatoid factor (RF): It is defined as an antibody against an antibody. RF and IgG join to from immune complexes that contribute to the disease process. Although predominantly encountered as IgM, rheumatoid factor can be of any isotype of immunoglobulins, i.e. IgA, IgG or IgM. RF was found at high frequencies in patients with RA (70-90%) [(19)].

(B) Anti-A2/anti-RA33 antibodies: Anti-A2/anti-RA33 auto-antibodies occur in approximately one-third of RA patients but are also present in SLE and mixed-connective tissue disease. Depending on the control groups, specificities between 90%-96% have been described for anti-RA33 in RA [(11)].

(C) Anti-citrullinated peptide/protein antibodies (ACPA): Anti-citrullinated peptide/ protein antibody is the collective term for autoantibodies directed against citrullinated epitopes of proteins or synthetic peptides. Since their first description in 2000, numerous studies have proven their diagnostic value in RA [(20)].

(D) Anti-peptidylarginine deiminase 4 antibodies (anti-PAD4) enzyme: This enzyme catalyzes citrullination of polypeptides and has been detected in inflamed RA synovium [(21)].

Regulation of secretion:

It is only partially understood, the adrenergic system through beta 3-receptor stimulation on adipocyte decreases leptin gene expression. Similarly, the use of isoproterenol, a beta-agonist, is associated with a reduction in leptin levels. Modulation through the adrenergic receptor may explain leptin's decrease in response to cold, fasting and exercise [(21)].

Role of leptin in different physiological functions:

Physiological Role of Leptin in the Regulation of Neuroendocrine Function [(22)]:

Hypothalamic-pituitary-gonadal axis: Recent studies have indicated that leptin regulates reproductive function by activating neurons that project afferent input to GnRH neurons in the preoptic area and other hypothalamic areas [(23)].

Hypothalamic-pituitary-thyroid axis: Leptin directly stimulates TRH-expressing neurons in the paraventricular nucleus (PVN) of the hypothalamus to upregulate pro TRH gene expression. Leptin also indirectly influences TRH neurons in the PVN through signals from the arcuate nucleus, as melanocortins (induced by leptin) stimulate the thyroid axis [(24)].

Hypothalamic-pituitary-growth hormone axis: Children with the leptin receptor mutation can experience an early growth delay with subnormal concentration of growth horGH, IGF-1, and insulin' like growth factor-binding protein-3 (IGFBP3) [(24)].

Hypothalamic-pituitary-adrenal axis: Leptin causes a dose-dependent stimulation of CRH release in vitro. However, studies in humans with mutations in the leptin or leptin receptor genes revealed that despite abnormal leptin function, normal adrenal function is maintained [(25)].

Physiological Role of Leptin in the Regulation of Insulin Sensitivity:

Several studies have reported that leptin increases glucose uptake in isolated soleus muscle in vivo and in animal models. Leptin exerts insulin-like effects on skeletal muscle. Leptin also directly affects glucose metabolism in the liver, which seem to be one of the primary tissues where leptin acts [(12)].

Physiological role of leptin in the regulation of immune function:

Hypoleptinemic states are associated with increased risk of infection. A variety of immune cells express obRb, and it is likely that leptin directly affects immune function. In ex vivo studies, leptin has been shown to enhance phagocytic activity in macrophages, promote production of proinflammatory cytokines such as TNF-α, IL-6, and IL-12. It stimulates chemotaxis in polymorphnuclear cells.

In addition, we have shown that leptin promotes lymphocyte survival in vitro by suppressing Fas-mediated apoptosis. Overall, leptin promotes TH1 cell differentiation and cytokine production. The role of leptin in immune function also seems to be a permissive one in humans. Leptin-deficient children develop infections early in life, and many die because of these abnormalities. Leptin exogenously administered to children with congenital leptin deficiency greatly improves their immune function [(26)].

Physiological role of leptin in regulation of bone metabolism:

Leptin affects bone metabolism through both central and peripheral pathways. Leptin may mediate cortical bone formation by regulating the expression of several hypothalamic neuropeptides [(27)].

Leptin deficiency and leptin therapy in human disease:

Leptin replacement has been investigated for its physiological role in several conditions characterized by leptin deficiency. The main conditions that have been studied are congenital leptin deficiency, lipodystrophy, hypothalamic amenorrhea, and weight loss [(28)].

1- Congenital leptin deficiency: Congenital leptin deficiency is a rare autosomal recessive disease caused by mutations in the leptin gene. In addition to marked obesity mainly due to hyperphagia. Congenital leptin deficiency is associated with inadequate secretion of GnRH. Individuals with congenital leptin deficiency have a greater incidence of infection than the general population due to decreased proliferation and function of CD4 T cells. Leptin replacement reverses several of the changes seen with congenital leptin deficiency [(24)].

2-Hypothalamic Amenorrhea:

Hypothalamic amenorrhea is a common cause of absent menstrual periods and deficiency, such as those who exercise vigorously or have a low body fat mass such as in anorexia nervosa. These women are hypoleptinemic [(28)]**.** Leptin replacement may be a promising treatment for infertility in women with hypothalamic amenorrhea [(29)]**.**

Role of leptin in rheumatoid arthritis:

The neuroendocrine and immune systems communicate bi-directionally through common ligands and receptors during stress responses and

inflammation, and control cellular immune responses in several pathological situations including immune-inflammatory rheumatic diseases[30]. Although precise cause of RA is not known, various proinflammatory cytokines including TNF-alpha, interleukin-1, and interleukin-6 have been recognized as etiological factors. Increased leptin levels during infection and inflammation support the fact that leptin is a component of cytokine network, which mediates immune response [11].

Leptin plays an important role in the T-cell related inflammatory process and reportedly modulates T-helper cell activation in cellular immune response. Leptin activates monocyte/macrophage cells and increases the production of proinflammatory cytokines as TNF-alpha and interleukin-6. Besides, it enables conversion of T-cells into Th1 phenotype and release of interleukin-2 and interferon gamma [31].

Leptin also modulates both innate and adaptive immunity. As described above, leptin activates the proliferation and phagocytosis of monocytes and /or macrophages and regulates the cytotoxicity of NK cells. In neutrophils, leptin modulates chemotaxis. Regarding adaptative immunity, leptin induces proliferation of naïve T cells, promotes TH1 cell immune responses and downregulates TH2 cell immune responses. Leptin is also able to modulate the activity of T reg cell, which are potent inhibitors of autoimmunity [32].

Therefore, leptin can be considered as a link among immune tolerance, metabolic function and autoimmunity. Strategies aimed at interfering with leptin signaling could provide future innovative therapies for autoimmune disorders such as RA. Since the discovery of leptin in 1994 by the group led by Jeffrey Friedman, white adipose tissue was considered only an energy storage tissue [33].

In the past decade, white adipose tissue has been recognized to be a very active tissue and an unexpected source of bioactive peptides, termed adipokines. In addition to their metabolic activities, it is now well known that these adipose-derived factors represent a new family of compounds that could participate in several processes including inflammation and immunity. Most immune cell types express LEPR at their surface, which suggests a role for-leptin in immune responses. Currently, leptin could be considered a link between the neuroendocrine and immune systems [7].

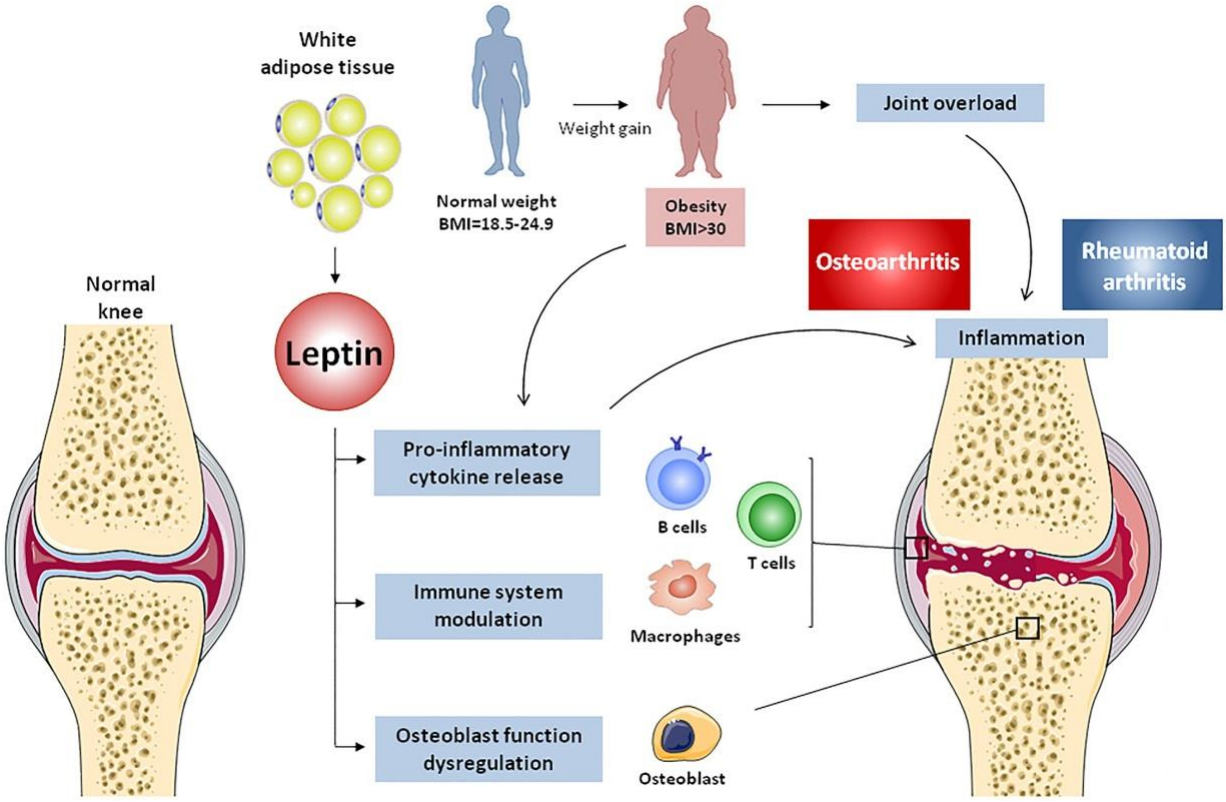

Figure (1): Schematic representation of the effects of adipose tissue-derived leptin on RA [34].

Role of leptin in regulation of innate immune responses:

Leptin and NK cell:

The involvement of leptin in NK cell activity was first discovered when obese lepr-deficient mice showed impaired NK cell activity. Subsequently, it was demonstrated that NK cells express the long-form of LEPR. The development of leptin resistance in obesity has been widely acknowledged, so that leptin resistance could explain the lack of leptin responsiveness in NK cells after long-term exposure. Accordingly, obese individuals have been widely acknowledged, so that leptin resistance could explain the lack of leptin responsiveness in NK cells after long-term exposure. Accordingly, obese individuals have been found to have lower NK cell count and function than lean individuals. After weight loss, plasma leptin levels are decreased and LFN_Y production by NK cell is increased **(35).**

Leptin and neutrophils: LEPR has been detected in human polymorphnuclear neutrophils, although these cells only express the short from of LEP. The short form of LEPR lacks most of the intracellular domain of the receptor and its functional activity is not yet fully understood. However, it might have signaling capability through mitogen-activated protein kinase (MAPK) signaling, but not through JAK-STAT signaling pathways as the long–form does**.** Leptin acts as a stimulatory mediator on neutrophils, as it activates chemotaxis and oxidative function. At the same time, it can also inhibit neutrophil chemotaxis to classical neutrophilic chemo-attractants, viap38 MAPK and Src kinases **(35).**

Leptin and eosinophils and basophils: Human eosinophils also express the long- and short- forms of LEPR on the cell surface. Treatment with recombinant leptin in vitro delays eosinophils apoptosis, implying that leptin can act as a survival factor, as it did in aforementioned neutrophils **(35).**

Leptin and monocytes and macrophages:

Both the long- and short-isoforms of LEPK are expressed in mouse and human macrophages. The first evidence of leptin effects on monocytes was reported in 1999 in vitro. Since then, it is well established that leptin promotes activation and proliferation of circulating monocytes, induces the production of proinflammatory cytokines by monocytes, stimulates the oxidative burst and finally, can enhance chemotactic responses **(3).**

Role of leptin in adaptive immune responses:

The first evidence for a role for leptin in adaptive immunity was reported by **kimura and co-workers (9)**. They observed that lepr- deficient obese mice showed thymus atrophy and T-cell lymphopenia. Since then, several studies have described key leptin actions on T cell and B cell populations.

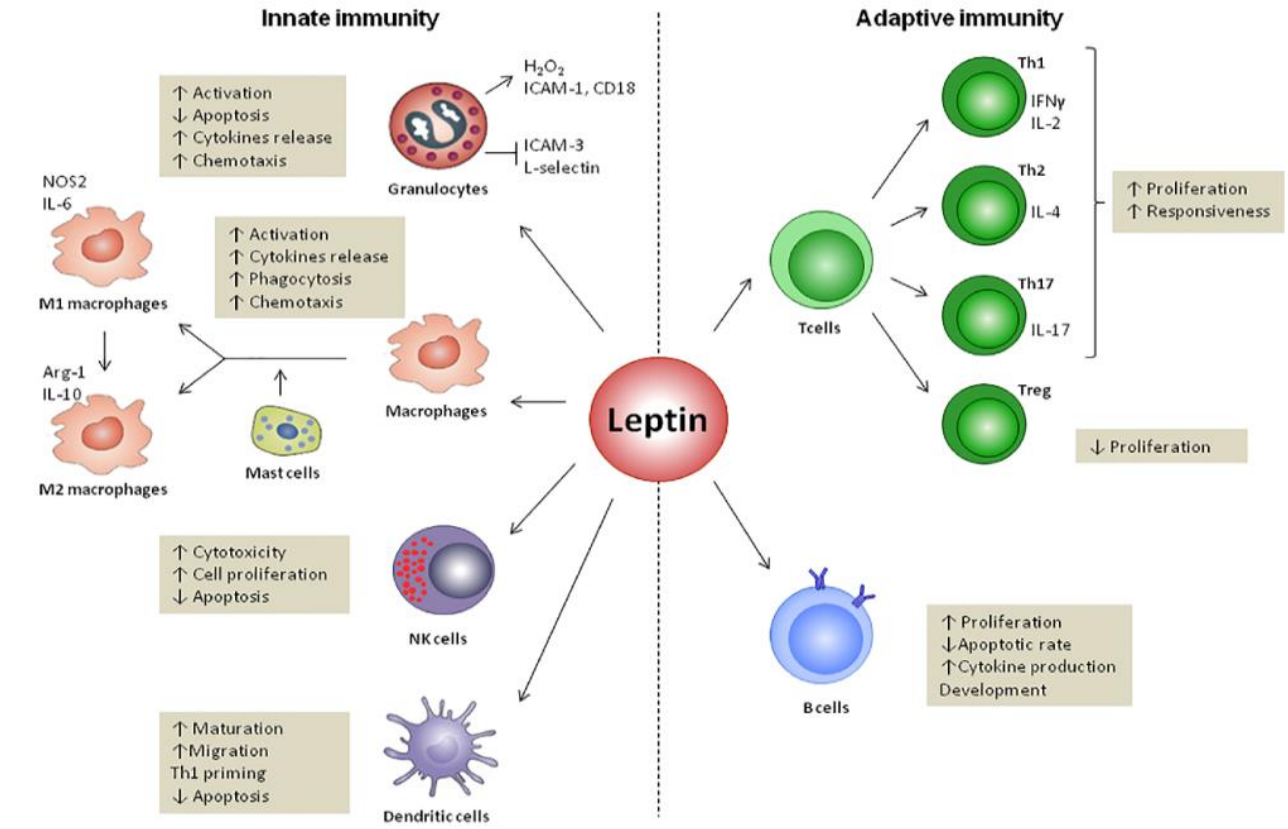

Figure (2): Leptin effects on innate and adaptive immunity **(34).**

Assessment of rheumatoid arthritis activity:

Acute phase reactants: Acute phase reactant levels, particularly the ESR and CRP, constitute the most objective disease activity measures. Acute phase reactant levels correlate well with both clinical disease activity measurements and radiographic progression of joint damage [(33)].

Disease activity score (DAS): The basis of the DAS was the clinician's decision to raise or lower DMARD doses based on a largely qualitative assessment of disease activity and reflected clinical practice in about 1990 [(35)].

DAS28 score: A modification of the DAS is considerably more practical. The DAS28 eliminated the grading of joints and reduced the number of joints evaluated to 28. It has largely replaced the traditional DAS in clinical trials and in practice [(32)].

Relation between disease activity and serum leptin levels:

A significant association may exist between RA patients' risk of severity and leptin levels. Plasma leptin levels have been observed to be higher than in healthy controls. **Welt *et al.*** [(29)] reported that serum leptin levels were higher in patients with erosive RA.

Similarly, **Bokarewa *et al.*** [(33)] found higher serum leptin levels than synovial fluid in RA patients, but latter evidently dropped compared to the former in arthritis patients in the absence of joint erosion, suggesting that leptin consumption in the joints may be involved in protection against erosions, and therefore the leptin protects against bone erosion.

SUMMARY

RA is a multi-system disorder with joint inflammation, which causes patients chronic pain, disability and increased mortality.

Leptin was initially described, as a hormone that regulates food intake and energy balance and it is a mediator of the inflammatory process. Leptin is a master regulator of the immune system. Disequilibrium in circulating levels and dysregulation of its secretion by white adipose tissue as well as by other peripheral tissues, can impair immune function and the integrity of a correct immune response

Numerous data support the key role of leptin in immunity and autoimmune diseases. Prevailing studies suggest the leptin is of great importance in the pathogenesis of RA. Blocking the key signal pathways of leptin and inhibiting leptin activity, such as with leptin antagonists, may be a promising way for the therapeutic potential of RA at risk of detrimental effects. Hence, further understanding of the mechanism of leptin would be advantageous in the future in RA treatment.

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